Supporting text

Birth death processes on a mutation network. Let us consider a birth death process with mutations. There are n types of mutations, with rates $u_1, \ldots, u_n$. Each mutation event corresponding to rate u_i leads to a phenotype resistant to drug i. To develop resistance to all n drugs, a cell must accumulate n mutations. Simple combinatorics suggests that there can be $m = 2^n - 1$ different phenotypes, resistant to one or more drugs. In particular, there are $\left(\begin{array}{c} n \\ k \end{array} \right)$ phenotypes resistant to $k \leq n$ drugs. Fig. 1 illustrates the mutation network for $n = 3$.

Each phenotype can be labeled by a binary number where "1" indicates resistance to the drug corresponding to its position and "0" indicates susceptibility (Fig. 1). In particular, type A^m is resistant to all n drugs and corresponds to the binary label $s = m = 11 \ldots 1$ (repeated n times). We can set up a birth death process on this mutation network. Each phenotype A^s with some $0 \leq s \leq m$, has arrows coming in and out, see Fig. 5. We assume that in time interval Δt, the following events can occur with each phenotype A^s, $0 \leq s \leq m$:

- With probability $L_s(1-\sum_j u_j^{s,out})\Delta t$ a cell of type A^s reproduces, creating an identical copy of itself;
- For each outgoing arrow, with probability $L_s u_j^{s,out}$ a cell of type A^s reproduces with a mutation, creating a cell of type $\mathrm{A}_j^{s\,next}$, for all j;
- With probability $(D_s + H_s)\Delta t$, a cell of type A^s dies.

Note that we neglect the probability of double (simultaneous) mutations.

We start with M_0 cells of type A^0 and follow the process until the first cell of type A^m has been created. We would like to calculate the probability, $P_n(t)$, that at least one cell of type A^m exists at time t. Here, the subscript n refers to the number of drugs used.

Let us introduce the function $\xi_{i_0,\ldots,i_m}$, the probability to have i_s cells of type A^s, where $0 \leq s \leq m$ are binary numbers. We can write down the Kolmogorov forward equation,

$$\dot{\xi} = \sum_{s=0}^{m} Q\{A^s\}, \qquad \textbf{[1]}$$

where $Q\{A^s\}$ is the contribution from all events leading to the state $(i_0, \ldots, i_m)$,

$$\begin{aligned} Q\{A^s\} &= \xi_{\ldots i_s-1\ldots}(i_s-1)L_s\left(1-\sum_j u_j^{s,out}\right) + i_s L_s \sum_j \xi_{\ldots i_s \ldots i_j-1\ldots} u_j^{s,out} \\ &+ \xi_{\ldots i_s+1\ldots}(i_s+1)(D_s+H_s) - \xi_{\ldots} i_s(L_s+D_s+H_s). \qquad \textbf{[2]} \end{aligned}$$

We used the following shorthand notations: $\xi_{\ldots}$ stands for $\xi_{i_0,\ldots,i_m}$, and the only explicit subscripts indicate the indices that are different from $(i_0, \ldots, i_m)$.

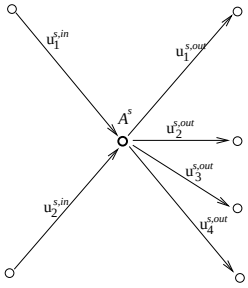

Figure 5: One vortex of a mutation diagram.

In Eq. **2**, the first term is faithful reproduction, the second term is all possible mutations, the third term is death, and the last term comes from the probability of no change.

Probability generating function. It is convenient to define the probability generating function, $\Psi(x_0, \ldots, x_m; t)$,

$$\Psi(x_0, \ldots, x_m; t) = \sum_{i_0,\ldots,i_m} \xi_{i_0,\ldots,i_m} \prod_{s=0}^{m} x_{\bar{s}}^{i_s}, \qquad \textbf{[3]}$$

where $\bar{s}$ denotes the binary number obtained from s by interchanging 0's and 1's. Note that $\Psi(0, 1, \ldots, 1; t)$ has the meaning of the probability that at time t, no cells of type A^m have been created. Then

$$P_n(t) = 1 - \Psi(0, 1, \ldots, 1; t).$$

Let us multiply Eq. **1** by $\prod_{s=0}^{n} x_{\bar{s}}^{i_s}$ and sum over all indices to obtain the equation for the generating function:

$$\begin{aligned} \frac{\partial \Psi}{\partial t} &= \sum_s \frac{\partial \Psi}{\partial x_{\bar{s}}} \left[x_{\bar{s}}^2 L_s \left(1 - \sum_j u_j^{s,out} \right) + D_s + H_s \right. \\ &+ \left. x_{\bar{s}} L_s \sum_j x_{\bar{j}} u_j^{s,out} - (L_s + D_s + H_s) x_{\bar{s}} \right]. \end{aligned} \qquad \textbf{[4]}$$

Note that the variables with the index $\bar{s}$ appear with the coefficients corresponding to type s, see also Fig. 1. The partial differential equation above can be solved by the method of characteristics. If we introduce the following shorthand notation,

$$u^{s,out} = \sum_j u_j^{s,out},$$

then the equations for characteristics are:

$$\dot{x_{\bar{s}}} = L_s \left(1 - u^{s,out}\right) x_{\bar{s}}^2 + \left[L_s \sum_j u_j^{s,out} x_{\bar{j}} - (L_s + D_s + H_s) \right] x_{\bar{s}}$$

$$+ \quad D_s + H_s, \quad 0 \le s \le m, \qquad \textbf{[5]}$$

and we have

$$P_n(t) = 1 - x_m(t)^{M_0}, \qquad \textbf{[6]}$$

where the function $x_m(t)$ is the solution of system **5** corresponding to the initial conditions

$$x_j(0) = 1, \quad 1 \le j \le n, \quad x_0(0) = 0. \qquad \textbf{[7]}$$

Two-phase computer simulations. To investigate the dynamics of resistance generation, we performed computer simulations of Eqs. **5**. In the absence of treatment, the parameter H_s was set to zero. In the presence of treatment, we switched on a nonzero value of H, which we took to be the same for all susceptible and partially susceptible phenotypes, and zero for the fully resistant phenotype. Note that system **5** simplifies significantly if we assume that all growth rates, death rates, and mutation rates are the same.

The probability of treatment failure under treatment with n drugs, P_n^{tot}, is given by $\lim_{t\to\infty} P_n(t)$. Let us suppose that treatment starts at time $t = t_*$. To find the probability of treatment failure numerically, we first need to solve system **5** under the treatment conditions (that is, with nonzero values of H), with initial conditions given by Eqs. **7**. We run the simulations until the values for $x_s(t)$ plateau. Then these limiting values are used as *initial conditions* for Eqs. **5** with *pretreatment* coefficients ($H = 0$). This simulation is performed for the length of time t_*. The resulting value for x_m is used to evaluate the probability of treatment failure.

The goal of the simulations is to find the probability of treatment failure starting from a tumor of a certain size, N. However, in the framework designed in the previous section, we cannot deterministically control the size of tumor. At time t_*, the size of the tumor is a random quantity with the mean $M_0 e^{(L-D)t_*}$ and variance $\sigma^2 \approx \frac{(L+D)}{2(L-D)} e^{2(L-D)t_*}$, where M_0 is the initial number of cells.

The uncertainty in size comes mostly from the early stages of growth where stochastic effects play an important role. Therefore, if we start the simulations from a sufficiently large number of cells (more precisely, $M_0 \gg \sqrt{\frac{L+D}{2(L-D)}}$), then with a high probability, the size of tumor at time $t = t_*$ will be close to $M_0 e^{(L-D)t_*}$. Let us suppose that we are interested in determining the size of the tumor for which the probability of treatment failure is $\delta \ll 1$. Then the error coming from the uncertainty in colony size can be estimated,

$$e_{size} = \frac{L+D}{4(L-D)} \left(\frac{\log \delta}{M_0} \right)^2 .$$

Another source of error is the process of mutation. By starting the simulation from $M_0 \gg 1$ wild type cells, we assume that no mutants have been generated in the first M_0 divisions. This leads to the error of the order

$$e_{mut} = \frac{u M_0}{\delta}.$$

As we can see, increasing M_0 decreases e_{size}, but it increases e_{mut}. There is an optimal number, M_0, which minimizes the total error. In general, large mutation rates ($u > 10^{-4}$) and low values of δ ($\delta < 10^{-3}$) cannot be handled by the method, because they lead to an error of $> 10\%$ even for $D = 0$.

Analytical results. In several special cases, analytical solutions for system **5** are possible. In particular, one can handle the case where we look at the treatment phase only. Let us start from M_0 nonmutant cells (no preexistence). We will make the following symmetry assumption: all the mutation, growth and death rates corresponding to different types are the same. Then we have the following equations:

$$\dot{x}_0 = Lx_0^2 - (L+D)x_0 + D, \quad \textbf{[8]}$$

$$\dot{x}_i = L(1-iu)x_1^2 + (iLux_{i-1} - (L+D+H))x_i + (D+H), \quad 1 \le i \le n, \quad \textbf{[9]}$$

where $P_i(t) = 1 - [x_i(t)]^{M_0}$. The initial conditions are

$$x_0 = x_i = 0.$$

We can calculate the limiting behavior as time goes to infinity. We have, under the assumption that $u \ll (L-D)$, that

$$\lim_{t\to\infty} x_0 = \frac{D}{L}, \quad \textbf{[10]}$$

$$\lim_{t\to\infty} x_i = 1 - \frac{i!(L-D)L^{i-1}u^i}{(D+H-L)^i}, \quad i > 0. \quad \textbf{[11]}$$

If the total number of drugs used is n, then the probability of treatment failure is

$$P_n^{\uparrow} = 1 - x_n^{M_0} \approx M_0 \frac{n!(L-D)L^{n-1}u^n}{(D+H-L)^n}.$$

Now, let us include the pretreatment phase and allow for generation of mutants before treatment starts. As described above, we first need to find the limiting values of x_i under the treatment conditions, which are given by formulas **10** and **11**. Then we use these as the initial conditions for the pretreatment equations, in the interval $0 \le t \le t_*$, where t_* is the time when treatment starts. The quantities $[x_i(t_*)]^{M_0}$ are the probabilities of treatment success with therapy starting at time t_*. We have,

$$\dot{x}_0 = Lx_0^2 - (L+D)x_0 + D, \quad \textbf{[12]}$$

$$\dot{x}_i = L(1-iu)x_1^2 + (iLux_{i-1} - (L+D))x_i + D, \quad i > 0, \quad \textbf{[13]}$$

and the initial conditions from **10— and 11. The equation for x_0 can be solved exactly to give**

$$x_0(t) = \frac{D}{L}.$$

The equation for x_1 is a Riccatti equation,

$$\dot{x}_1 = L(1-u)x_1^2 + (Du - (L+D))x_1 + D, \quad x_1(0) = 1 - \frac{(L-D)u}{D+H+L}.$$

We can find the exact solution and then take the limit $u \to 0$ to obtain an approximate formula for x_1,

$$x_1 \approx 1 - \left(\frac{He^{(L-D)t}}{H+D-L} - 1\right) u. \qquad \textbf{[14]}$$

Setting $e^{(L-D)t} = \frac{N_{treat}}{M_0}$, and neglecting 1 compared with this quantity, we find

$$P_1^{tot} \approx \frac{HN_{treat}u}{(H+D-L)}. \qquad \textbf{[15]}$$

If therapy is very strong such that $H \gg L - D$, we can see that treatment success does not depend on D.

We can find the size N corresponding to the probability of treatment failure, $\delta \ll 1$, from $x_1^{M_0} = 1 - \delta$, or

$$N = \frac{1}{u}\frac{(H+D-L)\delta}{H},$$

that is, the log size is a linear function of $|\log u|$ with slope $n = 1$.

Next, we examine the case of two drugs. We will make the approximation of a doubly stochastic process, whereby generation of one-hit mutants and their lineages is independent identically distributed stochastic processes. We will assume that the total population size changes according to the deterministic exponential law, $N(t) = M_0 e^{(L-D)t}$. Using the notion of filtered Poisson processes, we have,

$$P_2^{tot} = 1 - exp\left\{-2Lu\int_0^t N(t')(1 - x_1(t-t'))\,dt'\right\},$$

where $LuN(t')dt'$ is the probability to create a one-hit mutant in the time interval $(t, t+dt)$, and $1 - x_1(t-t')$ is the probability that the lineage resulting from that mutant will give rise to the production of a double mutant in the time from the creation of the lineage, t', to the current time, t. The factor 2 in the exponent comes from the two possibilities of acquiring two hits. The function x_1 is given in formula 14. The integral can be evaluated exactly, and then u can be neglected compared with 1. Note that quantity Nu is not assumed to be small. We obtain

$$P_2^{tot} = \frac{2Lu^2N}{L-D}\left(-\log\left[\frac{M_0}{N} + \frac{HLu}{(D+H-L)(L-D)}\right] - 1 + \frac{1}{N}\right);$$

this should be compared with formula 15 for the case of one drug.